

**VALIDITY OF AMNIOTIC FLUID LAMELLAR BODY  
COUNT AS A SCREENING TEST FOR FETAL LUNG  
MATURITY**

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## **CERTIFICATE**

This is to certify that this dissertation entitled “VALIDITY OF AMNIOTIC FLUID LAMELLAR BODY COUNT AS A SCREENING TEST FOR FETAL LUNG MATURITY” has been done by Dr. M.Maharani, post graduate in M.D (Obstetrics & Gynecology) under my overall supervision and guidance at Govt. Kasturba Gandhi Hospital, Madras Medical College, Chennai in partial fulfillment of the regulations of The Tamilnadu Dr. M.G.R Medical University for the award of the degree of M.D. OBSTETRICS & GYNECOLOGY in March 2007

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## **GLOSSARY**

AF	- Amniotic fluid
DPPC	- Dipalmitoyl phosphatidyl choline
FLM	- Fetal lung maturity
GDM	- Gestational diabetes mellitus
HMD	- Hyaline membrane disease
IUGR	- Intra uterine growth restriction
LBC	- Lamellar body count
L/S ratio	- Lecithin sphingomyelin ratio
NPV	- Negative predictive value
PPV	- Positive predictive value
PPROM	- Preterm premature rupture of membranes
RDS	- Respiratory distress syndrome
PET	- Preeclamptic toxemia
PG	- Phosphatidylglycerol
PI	- Phosphatidylinositol
µgm/ml	- micro gram per ml
µl	- micro liter
µm	- micro meter

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# INTRODUCTION

The primary objective in the management of many high risk pregnancies is to effect timely delivery in order to:

1. Prevent maternal morbidity and mortality.
2. Deliver a baby in an optimal condition and thereby minimizing perinatal morbidity and mortality.

In many high risk pregnancies, prematurity and consequent respiratory distress syndrome (RDS) remains a common cause of neonatal morbidity and mortality. Hence fetal lung maturity testing plays an important role in establishing obstetric strategy.

Respiratory distress syndrome (RDS) is an acute illness, usually of preterm infants, developing within 4-6 hours of birth.<sup>1</sup>

Approximately 2-3 percent of infants develop respiratory distress soon after birth. The overall incidence is 10-15%, but can be as high as 80% in neonate <28 weeks.<sup>2</sup>

The risk of RDS is inversely proportional to gestational age.<sup>3</sup> The introduction of treatments such as prenatal corticosteroids and prophylactic surfactant has reduced the rates of RDS at each gestational age.<sup>4</sup>

The accurate antenatal prediction of fetal lung maturity (FLM), based on results from amniotic fluid samples, is of utmost importance in the prevention of neonatal respiratory distress syndrome and its complications. The traditional as well as the current “gold standard” approach to such testing involves analysis of amniotic fluid (AF) by thin-layer chromatography to determine the lecithin-sphingomyelin ratio (L/S) and detect the presence of Phosphatidylglycerol. Although reliable, these chromatography methods are time consuming, costly, technique-dependent and not available at most institutions.

A number of alternatives have been evaluated; however each lacks sensitivity or specificity or is rendered invalid by contamination of amniotic fluid with blood or meconium. In **1989, Dubin<sup>5</sup>** described lamellar body count (LBC) in amniotic fluid through the use of widely available commercial cell counters.

This study was conducted to evaluate the validity of amniotic fluid lamellar body count as a screening test for fetal lung maturity.



## REVIEW OF LITERATURE

In the 1950s, it was discovered that the resistance of pulmonary alveoli to collapse during expiration was mainly caused by surfactants. Since then many tests were described to measure the surfactant level.

The lecithin /sphingomyelin ratio test, which was initially introduced by *Gluck et al*<sup>6</sup> in 1971 remains one of the most commonly used tests, and one of the standardized tests against which all other tests are compared.

*Donald et al*<sup>7</sup> found that of those infants with lecithin / sphingomyelin ratio of less than 2 at the time of delivery, the morbidity from RDS was 63% with an associated 23% mortality rate from RDS.

*Hallman et al*<sup>8</sup> found that the presence of Phosphatidylglycerol in the amniotic fluid indicates the pulmonary maturity and also found that the false positive rate was 1.8%. This rate is significantly lower than the false positive rate of L/S ratio which was about 5%.

*Torday et al*<sup>9</sup> compared the measurement of the L/S ratio to that of Saturated Phosphatidylcholine (SPC) in high risk pregnancies and found that the SPC is superior for the prediction of pulmonary immaturity.

*Clements et al*<sup>10</sup> found that the shake test was comparable to the L/S ratio and had a high predictive value for RDS when applied to uncontaminated amniotic fluid.

*Turner and Read et al*<sup>11</sup> found that the optical density at 650nm was a better predictor of fetal lung maturity.

In 1989, *Dubin* described a method for quantifying the lamellar body number density (lamellar body count) in amniotic fluid through the use of widely available commercial cell counters.

Several studies have demonstrated a high correlation between the lamellar body count and other methods used in fetal lung maturity testing.

1. A two years prospective clinical outcome study conducted by *Lee IS et al*<sup>12</sup> at the Department of Obstetrics and Gynecology, University of Ulsan, Korea to evaluate the usefulness of amniotic fluid lamellar body count (LBC) as a screening test for fetal lung maturity concluded that the LBC cut off value of 50,000/ $\mu$ l have the diagnostic sensitivity and specificity of 100% and 80% respectively.

2. The prospective study conducted by *Khazardoost et al*<sup>13</sup> at Tehran University of Medical Science, Iran to find out the sensitivity and specificity of LBC in amniotic fluid to predict fetal lung maturity concluded that the negative predictive value of LBC more than 50,000/ $\mu$ l was 92% and positive predictive value was 48% and sensitivity for prediction of RDS was 85% and specificity was 70%.
3. The majority of published reports used a COULTER brand of hematology analyzer. One study<sup>14</sup> used lamellar body counts from four different analyzers to assess fetal lung maturity. Of the four analyzers, the COULTER brand analyzer was found to be the best. The SYSMEX XE-2100 showed the best concordance (86%) with the coulter. The concordance of ADVIA 120 was 78%. Finally the concordance of the CELL-DYN 3500 with the coulter was 66%.
4. Lamellar body counts determined by light microscopy correlate well with results obtained for lamellar body counts using standard coulter counter techniques. (*Laura A Hunter et al*<sup>15</sup>)
5. *Ashwood et al*<sup>16</sup> reported that LBC is a good predictor of FLM, and they recommended a cut off of 50,000/ $\mu$ l as the decision threshold, with no cases of RDS above 48,000/ $\mu$ l.

6. ***Carlos et al*<sup>17</sup>**, conducted a prospective clinical outcome study among 130 women stated that LBC exceeding 30,000/ $\mu$ l predict pulmonary maturity correctly in all cases. He also reported that phospholipids analysis is not needed with LBCs >30,000/ $\mu$ l or <10,000/ $\mu$ l, but may be of benefit for values in the intermediate group.
7. ***Lewis PS, Lauria et al*<sup>18</sup>** conducted a study, and concluded that testing only specimens where LBC >8000/ $\mu$ l and <32,000/ $\mu$ l for the L/S ratio or phosphatidyl glycerol would preclude the need for 76% of all that assays.
8. ***Karcher R et al*<sup>19</sup>** conducted a study in 2005 and concluded that LBC and TDx FLM tests are equally accurate.
9. ***De Roche et al*<sup>20</sup>** conducted a study at Department of Obstetrics and Gynecology, Hartford Hospital, USA states that a lamellar body count of more than 37,000/ $\mu$ l correlated well with the L/S ratio and Phosphatidylglycerol value in pregnancies of diabetic patients.
10. ***Darlynn et al*<sup>21</sup>** had done a project to compare a FLM test, LBC in diabetic and non diabetic pregnancies and concluded that there was no statistically significant difference of the LBC between diabetic and non diabetic cases with the gestational ages (32-34weeks) studied.

11. A study<sup>22</sup> conducted in the Department of Obstetrics and Gynecology, Lithuania concluded that with a cut off of 28,000/ $\mu$ l the diagnostic accuracy for LBC as follows: Sensitivity 95.2%, specificity 88%, positive predictive value 64.5% and negative predictive value 98.8%.
12. *LDE Wijnberger et al*<sup>23</sup> done a meta analysis of six studies reported that the performance of both L/S ratio and LBC are equal in predicting RDS.
13. *Shohreh Bahasadri et al*<sup>24</sup> conducted a study in Tehran concluded that using the cut off of 10,000/ $\mu$ l and 45,000/ $\mu$ l LBC can serve as the first screening test for FLM.
14. *Geross, FN Bever et al*<sup>25</sup> done a cost effective analysis by comparing LBC and L/S ratio in 2002 concluded that LBC efficacy is equal to L/S ratio, and a cascade approach results in 80% savings to the hospital if the L/S ratio and percentage phosphatidyl glycerol are not done.

## **RESPIRATORY DISTRESS SYNDROME (RDS)**

Respiratory distress syndrome of the newborn is otherwise called as Hyaline Membrane Disease (HMD) which is characterized by grunting, intercostal retraction, nasal flaring, cyanosis in room air and requirement of oxygen to maintain adequate arterial oxygen pressure.

### **Incidence:**

HMD occurs in more than 50% of babies born before 28 weeks of gestation, but only in less than 30% of those born between 32 and 36 weeks<sup>2</sup>.

### **Pathophysiology:**

Respiratory distress syndrome occurs because of inadequate production of pulmonary surfactant by the type II alveolar cells of the newborn.

The surfactant spreads in the lung tissue – air interface and differentially reduces the surface tension of the alveoli leading to stability of the alveoli there by preventing alveolar collapse during expiration and allowing the alveoli to open easily at the next inspiration.

### **Surfactant:**

There are two types of alveolar epithelial cells.

Type I cells contain less subcellular organelles and these cells spread out thinly along the alveolar walls and comprise the alveolar epithelium.

Type II cells contain abundant mitochondria, endoplasmic reticulum, golgi apparatus and osmophilic lamellar bodies that contain surfactant.

Surfactant production starts in the 20<sup>th</sup> week of gestation and attains maximum level at 35<sup>th</sup> week of gestation<sup>4</sup>.

### **Surfactant Composition<sup>29</sup>:**

The pulmonary surfactant is a heterogeneous mixture of phospholipids (80%), proteins (10%) and neutral lipids (10%).

Dipalmitoyl phosphatidylcholine (DPPC) also referred as lecithin is the main component of the pulmonary surfactant.

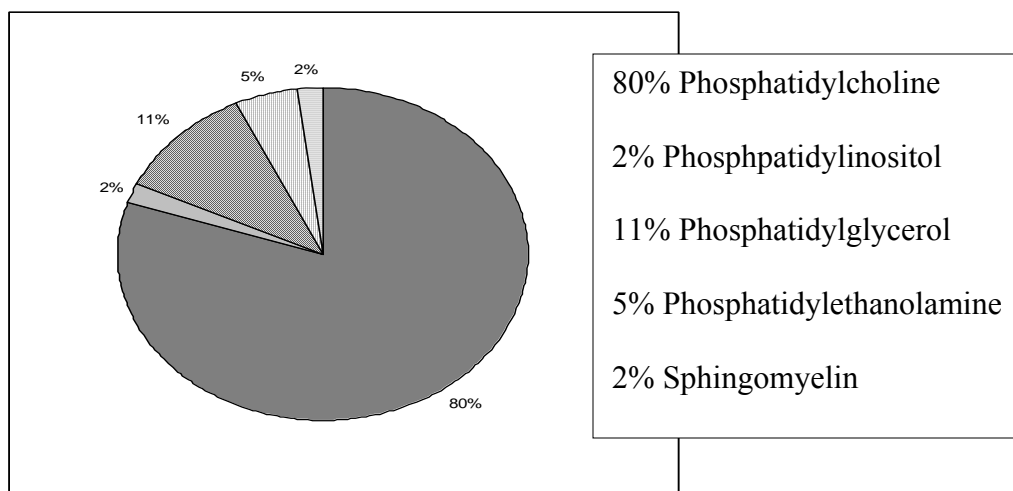


FIGURE 1. Glycerophospholipid composition of mature surfactant.

In addition to the phospholipid components of surfactant, recently four surfactant related proteins have been described. They are

1. Surfactant Protein A (SP-A)
2. Surfactant Protein B (SP-B)
3. Surfactant Protein C (SP-C)
4. Surfactant Protein D (SP-D)

### **Surfactant Pathway**

After its synthesis DPPC accumulates in osmophilic structures called lamellar bodies. The lamellar bodies are released from the cells into alveolar fluids, from there they go into the amniotic fluid. This makes it possible to assess the biochemical maturation of the fetal lungs by studying the amniotic fluid phospholipid composition.

### **Pathology**

In HMD, on macroscopic examination, the lungs appear deflated, have the consistency of liver and do not float in water.

Microscopically there are homogeneous eosinophilic membrane (Hyaline membrane) plastering the alveolar ducts and the terminal bronchioles. Other characteristic pathological changes are small fragments of basophilic material within the eosinophilic membrane, capillary congestion and lymphatic dilatation.



## **Aetiology**

Factors predisposing to RDS are:

### **Maternal factors**

Diabetes

Hypertension

Multiple Pregnancy

Malnutrition

Familial Disposition

### **Perinatal factors**

Premature delivery

Cesarean section

Male gender

Birth asphyxia

Hypothermia

Hemolytic disease of the new born

## **Prematurity**

Inadequate production of surfactant is the cause of RDS in prematurity.

## **Cesarean Section**

Cesarean Section increases the risk of neonatal respiratory distress twofold for elective procedures and tenfold for emergency procedures<sup>30</sup>. The reason for this increased risk of respiratory morbidity following cesarean section is probably a combination of delayed removal of lung fluid and exaggerated pulmonary hypertension.

## **Male Gender**

The delay in producing mature surfactant in male fetuses may be due to an androgen effect on type II pneumocytes<sup>30</sup>.

## **Birth Asphyxia**

During asphyxia, lung perfusion falls to very low levels, causing ischemic damage to pulmonary capillaries which in turn leads to leakage of protein rich fluid from damaged capillaries into the alveoli where surfactant will be inactivated.

## **Hemolytic Disease of the Newborn (HDN)**

A possible mechanism is via elevation of insulin levels due to  $\beta$ -islet cell hypertrophy. In the presence of severe erythroblastosis with anaemia, heart failure, hydrops fetalis surfactant inactivation can occur.

### **Maternal Diabetes**

Insulin has been shown to delay the maturation of type II pneumocytes with delayed appearance of phosphatidylglycerol and decrease the proportion of saturated phosphatidylcholine in surfactant.

### **Maternal Hypertension**

Maternal hypertension increases the risk of RDS, probably as a result of preterm delivery by elective cesarean section before the onset of labor.

### **Multiple Pregnancy**

In twin pregnancies, the second twin is usually at greater risk of developing RDS. It is not clear whether this increased risk is due to delayed maturation of the lungs or an increased risk of asphyxia in the second twin.

### **Malnutrition**

Deficiency of inositol might be important in these cases and supplementation of preterm infants has been shown to promote maturation of surfactant phospholipids.

### **Familial Predisposition**

Familial RDS is due to congenital deficiency of SP-B.

## **Factors protecting against RDS**

1. Antenatal Corticosteroids
2. IUGR
3. Premature rupture of membranes (PROM)
4. Maternal drugs and smoking
5. Female gender
6. Ethnicity

### **1. Antenatal Corticosteroids**

Meta analysis of all randomized controlled trials (RCT) suggests an approximate 50% reduction of risk of RDS and a 40% reduction of neonatal mortality<sup>31</sup>.

### **2. IUGR**

A stressful intrauterine environment might increase the production of glucocorticoids and catecholamines in fetal plasma, leading to earlier maturation of the fetal lungs and reduced risk of RDS.

### **3. PROM**

PROM over a short period of time decreases the RDS as a result of stress hormones and increases the production of surfactant.

#### **4. Maternal Drugs and Smoking**

Maternal narcotic addictions, cocaine use, smoking and alcohol intake all reduces the incidence of RDS in preterm babies.

#### **5. Gender**

Girls have less RDS at each gestational age compared with boys.

#### **6. Ethnicity**

Black infants have a lower incidence of RDS than white infants.

### **CLINICAL SIGNS**

The clinical signs (Respiratory distress scoring by Silverman) and the radiological criteria for the diagnosis of RDS are as below:

#### **RESPIRATORY DISTRESS SYNDROME SCORING BY SILVERMAN<sup>4</sup>**

<b>Score</b>	<b>0</b>	<b>1</b>	<b>2</b>
Respiratory rate	<60/m	60-80/m	>80/m
Cyanosis	None	None / 40% O <sub>2</sub>	Requires >40% O <sub>2</sub>
Chest retraction	None	Mild	Moderate to Severe
Grunting	None	Audible with stethoscope	Audible without stethoscope
Air entry	Good	Decreased	Barely audible

Score of 0-3 – mild RDS

4-6 – moderate RDS

7-10 – severe RDS

X-ray picture suggestive of RDS are

1. Granular pattern
2. Ground glass appearance
3. Air bronchogram

## **PREVENTION**

Antenatal corticosteroids have been proved to decrease the incidence of RDS. A meta analysis has shown that prophylactic surfactant therapy, that is given within 10-15 minutes of birth, leads to reduced mortality for infants of 31 weeks of gestation or less<sup>4</sup>.

## **TREATMENT**

1. The baby should be placed in the intensive neonatal care unit. Warmth, humidified oxygen, correction of hypovolaemia, correction of electrolyte imbalance, prevention of infections, maintenance of nutrition are the mainstay of the treatment.
2. Surfactant Therapy – it has significantly improved the outcome of infant with HMD.
3. Mechanical Ventilation is needed for severe RDS.

## **PROGNOSIS**

About one third of the babies may die. In mild affection with good vigor the baby may survive<sup>3</sup>.

## **SPECIFIC TESTS FOR THE ASSESSMENT OF FETAL LUNG MATURITY**

### **A. Biochemical quantitation of pulmonary surfactant**

Lecithin / Sphingomyelin Ratio (L/S ratio)

Phosphatidyl glycerol (PG)

Lung Profile

Saturated Phosphatidylcholine (SPC)

Microviscosimeter

Fluorescent polarization (surfactant / albumin ratio)

Surfactant – associated proteins

### **B. Biophysical measurement of surfactant function**

Shake test

Foam stability index

Tap test

### **C. Evaluation of amniotic fluid turbidity**

Visual inspection

Optical density at 650nm

Lamellar body counts

## **BIOCHEMICAL QUANTITATION OF PULMONARY SURFACTANT**

### **1. Lecithain / Sphingomyelin Ratio (L/S ratio)**

The L/S ratio test, which was initially introduced by *Gluck et al*<sup>6</sup> in 1971 remains the “gold standard” test.

The total phospholipids in amniotic fluid increase throughout gestation and there is a sharp increase at 35 weeks. Concentration of lecithin and sphingomyelin are equal until 35 weeks of gestation, when there is an increase in lecithin concentration, to almost four times that of sphingomyelin. Sphingomyelin is not related to maturation event.

The L/S ratio for normal pregnancy is less than 0.5 at 20 weeks gestational age and gradually increases to a value of 1 at about 32 weeks gestational age. A value of 2 was achieved by 35 weeks gestational age<sup>6</sup>.

RDS is very unlikely if the L/S ratio is more than 2<sup>32</sup>.

False positive rate is 2.7%.

False positive rate in Diabetic mothers with L/S ratio more than 2 is 7.4%

### **Disadvantages:**

1. Both blood and meconium staining of amniotic fluid is found to interfere with L/S ratio determinations<sup>33</sup>.
2. Requirements for expensive equipment and well trained personnel.
3. Time consuming.



4. Costly.
5. Not available in many smaller hospitals.

### **1. Phosphatidylglycerol and phosphatidylinositol**

Phosphatidylglycerol and phosphatidylinositol comprise smaller fractions of surfactant. Phosphatidylinositol peaks at 35 weeks gestation and dramatically decreases as term approaches<sup>8,34</sup>. In contrast, Phosphatidylglycerol is barely detectable until 35 weeks gestation, when it first appears. Then its concentration increases with gestation. Thus presence of Phosphatidylglycerol in amniotic fluid indicates fetal lung maturity.

Phosphatidylglycerol may not be as gestational age dependent as L/S ratio.

False positive for Phosphatidylglycerol determination was 1.8%.

Phosphatidylglycerol determination originally required thin layer chromatography, trained personnel and expensive equipment. But with the development of rapid slide agglutination test, termed as Amniostat FLM (AFLM), Phosphatidylglycerol estimation has become less expensive, faster and could be performed by untrained personnel. Phosphatidylglycerol concentration more than 2µgm/ml or 2% of total phospholipids indicates fetal lung maturity.

### **3. LUNG PROFILE**

The lung profile includes the L/S ratio, desaturated lecithin, Phosphatidylglycerol and phosphatidylinositol concentrations. The false positive rate was reduced to less than 1% with the combined lung profile test<sup>34</sup>.

### **4. SATURATED PHOSPHATIDYLCHOLINE**

Saturated Phosphatidylcholine in amniotic fluid is found to be superior to L/S ratio for prediction of pulmonary immaturity. The cut off value of Saturated Phosphatidylcholine more than 500µgm/dl indicates fetal lung maturity<sup>9</sup>. In addition the Saturated Phosphatidylcholine was found to be valid in the presence of blood and meconium. A significant draw back is the requirement of thin layer chromatography and skilled personnel.

### **5. MICROVISCOSIMETER**

Microviscosimeter testing measures surfactant associated with a phospholipid membrane using florescent dye techniques.

### **6. FLUORESCENT POLARIZATION (SURFACTANT / ALBUMIN RATIO)**

A recently introduced TDx FLM assay is an automated fetal lung maturity test based on the principle of fluorescent polarization. It only requires approximately 1ml of amniotic fluid. The TDx FLM assay provides a quantitative, rapid and

automated measurement of the amniotic fluid surfactant to albumin ratio. A surfactant to albumin ratio of 50-70 mg surfactant /gm of albumin has been considered mature in most studies<sup>35</sup>.

## **Biophysical measurement of surfactant function**

### **1. Shake Test And Foam Stability Index.**

These two tests use the principle that when ethanol is added to amniotic fluid, the non surfactant foam causing substances in amniotic fluid are removed. Any stable foam layer that persists after shaking is due to the presence of surfactant in a critical concentration. When the shake test predicts maturity, its accuracy is close to 100%, but a negative test is not a good predictor of pulmonary immaturity<sup>10</sup>.

#### **Foam stability index**

This is a semi-quantitative test. In this test the fluid is mixed with ethanol in the necessary amounts to achieve alcohol concentrations ranging from 44% to 50%.

A positive foam stability index at an ethanol concentration of 47% is a better predictor of fetal lung maturity<sup>36</sup>.

### **2. Tap Test**

This test is performed by mixing 1ml of amniotic fluid with one drop of 6N hydrochloric acid and then adding 1.5ml of diethyl ether. The test tube is

briskly tapped creating bubbles in the ether layer. If the fetus is mature, the bubbles will rise to the surface and break down. If the fetus is immature, bubbles are stable or break down slowly<sup>37</sup>.

Similar to other tests, it has positive predictive value 98% to 100%, but the negative test is not a good predictor of fetal lung maturity.

## **Evaluation of Amniotic Fluid Turbidity**

### **1. Visual Inspection**

Visual comparison of the turbidity of unspun amniotic fluid against positive (mature) or negative (immature) controls predicts the fetal lung maturity and immaturity with good sensitivity and specificity<sup>38</sup>.

### **2. Optical density at 650nm**

The measurement of the amniotic fluid optical density at 650nm value between 0.1 and 0.2 predicts fetal lung maturity<sup>11</sup>.

### **3. Lamellar body count**

Lamellar bodies are concentrically layered structures produced by type II pneumocytes, which are extruded into the lung fluid and can enter the amniotic fluid via the fetal mouth.

They are comprised of phospholipids that represent the storage form of surfactant and have a similar size to platelets, ranging from 1 to 5µm in diameter<sup>14</sup>.

Therefore, by performing a “platelet count” on amniotic fluid, a count of lamellar bodies (LBC) will be obtained by using automated cell counter.

Amniotic fluid can be collected by the following methods:

1. Amniocentesis
2. During cesarean section.
3. Vaginal pool specimen composed of free flowing amniotic fluid (Not contaminated with mucous).

Minimum of 1ml is required. The uncentrifuged specimens are used for lamellar body count.

Recently, a consensus LBC protocol<sup>14</sup> was published and a fetal lung maturity was suggested by a cut off of 50,000/ $\mu$ l or greater and immaturity was suggested by a count of 15,000/ $\mu$ l or lower regardless of the hematology analyzer and also states that centrifugation is not a necessary step and should be abandoned.

Coulter type of hematology analyzer is commonly used, but sysmex also gives almost equal results. Many studies proved that lamellar body count correlates positively with advancing gestational age and its efficacy is comparable to L/S ratio and phosphatidyl glycerol estimation.

Lamellar body count determined by light microscopy correlates well with the results obtained from Coulter counter.

**The advantages are**

1. It is rapid, simple, more precise, more objective, inexpensive and universally available.
2. Meconium and lysed blood will not interfere with LBC. However EDTA (ethylene diamino tetra acetic acid) treated blood may artificially decrease the count.<sup>33</sup>
3. Vaginal pool specimens composed of free flowing amniotic fluid will be usable<sup>6</sup>. (Those vaginal pool specimens contaminated with mucous cannot be tested)
4. In diabetic patients, LBC is more reliable than L/S ratio<sup>20</sup>.

## **AIM OF THE STUDY**

To evaluate the efficacy of amniotic fluid lamellar body count (LBC) as a screening test for fetal lung maturity.

# **MATERIALS AND METHODS**

## **STUDY DESIGN**

A prospective clinical outcome study for assessing the efficacy of amniotic fluid lamellar body count as a screening test for fetal lung maturity.

## **PLACE OF STUDY AND STUDY POPULATION**

This study was conducted over a period of one year, (July 2005 to June 2006) among the patients admitted in the Government Kasturba Gandhi Hospital, Madras Medical College at Chennai.

Total number of 115 cases were included in the study using certain inclusion criteria. The study group consisted of patients irrespective of age, parity, booking status and socioeconomic status with risk factors for RDS.

Amniotic fluid lamellar body count from 115 pregnant women between 28 weeks and 40 weeks of gestation were evaluated. The patients with the confounding factors which would have their own influence on respiratory distress syndrome were excluded from the study even at that point of time. So, the results could be derived with only 100 patients in this study population.



## **INCLUSION CRITERIA**

1. Preterm labor.
2. Premature rupture of membranes. (PROM)
3. Preeclamptic toxemia. (PET)
4. Repeat cesarean section.
5. Diabetes.
6. Intra uterine growth restriction. (IUGR)

## **EXCLUSION CRITERIA**

1. Birth asphyxia.
2. Meconium stained liquor.
3. Sepsis.
4. Jaundice.
5. Patients delivered after steroid treatment.

## **METHODS OF STUDY**

Amniotic fluid was collected by the following methods:

1. Amniocentesis (under USG guidance, after getting informed consent, trans abdominal amniocentesis was performed).
2. During cesarean section.

3. Free flowing amniotic fluid (not contaminated with mucous) flowing through the cervical os

Minimum of 1ml was collected, refrigerated. Lamellar body count was obtained from the uncentrifuged specimens by using platelet channel of commercial cell counter (Sysmex). A balanced electrolyte solution was aspirated at least once into the instrument to prime it and to remove any remaining blood that might have been left on the tip from previous analyses. Then the amniotic fluid specimens were aspirated into the Sysmex counter, the platelet channel measures the number and size of the particles that are 1-5 $\mu$ m in diameter which corresponds to lamellar body counts.

Recently a consensus lamellar body count protocol<sup>14</sup> was published, and a fetal lung maturity cut off of 50,000/ $\mu$ l was suggested with out discussion regarding the hematology analyzer used. The Lamellar body count of 50,000/ $\mu$ l was chosen as a cut off point for the fetal lung maturity.

After delivery, each infant was evaluated for any evidence of respiratory distress syndrome. Standard clinical and radiographic criteria were used to diagnose respiratory distress syndrome.

The variables studied included examination of the preterm infant with specific reference to

1. Sex/weight/apgar score (1 minute apgar)
2. Gestational age
3. Evidence of respiratory distress syndrome and score by Silverman
4. Evidence of
  - a. Hypoglycemia
  - b. Jaundice
  - c. Hypothermia
  - d. Birth asphyxia
  - e. Sepsis
  - f. Bronchopneumonia
5. In case of infant death, time and cause of death were noted.

When respiratory distress was present for the baby, an x-ray chest was taken as the chief investigation.

X-ray picture of

4. Granular pattern
5. Ground glass appearance
6. Air bronchogram

were taken as evidence of Idiopathic respiratory distress syndrome.

## RESULTS AND ANALYSIS

Out of a total of 115 patients studied initially, 100 patients were included in the study finally.

**TABLE 1. AGE DISTRIBUTION**

**n=100**

Sl.No	Age Group(yrs)	Number of Patients	% of Patients
1	$\leq 20$	12	12
2	21-25	53	53
3	26-30	30	30
4	31-35	5	5
5	$\geq 36$	0	0

- In this study majority of the patients were in the age group of 21-30yrs, which accounts for 83%, and none of the patients were above 35yrs.

**TABLE 2. BOOKING STATUS**

<b>Sl.No</b>	<b>Booking Status</b>	<b>Number of Patients</b>	<b>% of Patients</b>
1	Booked	95	95
2	Unbooked	5	5

- 95% of the patients were booked, either in this hospital or in other hospitals.
- Effective antenatal care helps in proper screening of the patients and identifying the at risk patients for RDS.

**TABLE 3. SOCIO ECONOMIC STATUS**

<b>Sl.No</b>	<b>Socio Economical class</b>	<b>Number of Patients</b>	<b>% of Patients</b>
1	Class I	0	0
2	Class II	0	0
3	Class III	4	4
4	Class IV	13	13
5	Class V	83	83

- Majority of the patients belonged to class V socio economical class, since this hospital essentially caters to those below poverty line.

**TABLE 4. GRAVIDITY DISTRIBUTION**

<b>Sl.No</b>	<b>Gravidity</b>	<b>Number of Patients</b>	<b>% of Patients</b>
1	Primi gravida	52	52
2	Second gravida	29	29
3	Third gravida	15	15
4	Fourth gravida and above	4	4

- Primi gravida contributes to 52% of the study population, second and third gravida accounts for 44% and the gravida four and above accounts for only 4%.

**TABLE 5. RISK FACTORS**

<b>Sl.No</b>	<b>Risk factors</b>	<b>Number of Patients</b>	<b>% of Patients</b>
1	Preterm labor	44	44
2	Repeat cesarean section	17	17
3	Preeclamptic toxemia	16	16
4	Diabetes complicating pregnancy	10	10
5	PPROM	11	11
6	IUGR	2	2

- The various indications for assessing the lamellar body count are given in this table.
- In this study 44% of the patients had preterm labor without any other risk factors for RDS.
- In the above data pre term patients with other risk factors like PET, diabetes are not included in the pre term group and they are included in the PET, diabetes group.
- 17% of repeat cesarean section cases were also selected, since cesarean section is also one of the risk factors for RDS.
- 2 cases of IUGR (protecting factor) were also included.



**TABLE 6. GESTATIONAL AGE**

<b>Sl.No</b>	<b>Gestational Age (weeks)</b>	<b>Number of Patients</b>	<b>% of Patients</b>
1	28-31	18	18
2	32-35	57	57
3	36-40	25	25

- In this study, about 75% of the patients were with gestational age below 36 weeks.
- Patients with very pre term (<32 weeks) accounts for 18%, whose babies are more vulnerable for RDS.
- 25% of the patients with the gestational age of 36-40 weeks were selected to study the impact of lamellar body count on the gestational age, that is for internal comparisons.

**TABLE 7. MODE OF DELIVERY**

<b>Sl.No</b>	<b>Mode of delivery</b>	<b>Number of Patients</b>	<b>% of Patients</b>	<b>Number of cases of RDS</b>	<b>% of RDS</b>
1	Labor Natural	64	64	12	18.75
2	Cesarean Section	30	30	6	16.6
3	Forceps	4	4	-	-
4	Vacuum	2	2	-	-

- Two third (2/3) of the babies were delivered by labor natural.
- In addition to the 17 cesarean section cases (both elective and emergency) were selected initially, 13 emergency cesarean sections were performed.
- There was no statistical significant difference in the incidence of RDS between labor natural and cesarean section. (p value =0.8)

**TABLE 8 A. SIGNIFICANT LAMELLAR BODY COUNT**

Sl.No	LBC (per $\mu$ l)	Number of Patients	% of Patients
1	< 50,000	23	23
2	> 50,000	77	77

- In this study, the lamellar body count varied from 5,000/ $\mu$ l to 2,88,000/ $\mu$ l with a mean of 86,378/ $\mu$ l.
- In 23% of the patients lamellar body count was below 50,000/ $\mu$ l. Only in 5% of the patients the lamellar body count was <25,000/ $\mu$ l.

**TABLE 8B. LAMELLAR BODY COUNT**

Sl.No	Lamellar body count (per $\mu$ l)	Number of Patients	% of Patients
1	$\leq$ 24,999	5	5
2	25,000-49,999	18	18
3	50,000-99,999	50	50
4	$\geq$ 1,00,000	27	27

**TABLE 9. LAMELLAR BODY COUNT IN RELATION TO  
GESTATIONAL AGE.**

Sl.No	Gestational Age (weeks)	Total no. of Patients	LBC <50,000/ $\mu$ l		LBC >50,000/ $\mu$ l	
			Number	%	Number	%
1	28-31	18	10	55.6	8	44.4
2	32-35	57	13	22.8	44	77.2
3	36-40	25	0	0	25	100

- Out of the 18 patients in the gestational age of 28-31 weeks, the LBC was less than 50,000 in 10 signifying fetal lung immaturity.
- In the gestational age group of 32-35 weeks, the same was only 13 out of 57, signifying an increased lung maturity.
- All the 25 babies were fully mature in the GA 36-40 weeks.
- All these indicate that the lamellar body count increases, as the gestational age increases.

**TABLE 10 LBC of <50,000 /  $\mu$ l and RDS in Different Type of Cases.**

<b>RISK Factor</b>	<b>Total No. of Patients in each group</b>	<b>No. of Patients with LBC &lt;50,000/ <math>\mu</math>l</b>	<b>Percentage of Patients with LBC &lt;50,000/ <math>\mu</math>l</b>	<b>No of Cases of RDS</b>	<b>Percentage of Cases of RDS</b>
Preterm	44	12	27.3	6	13.7
PET	16	7	43.8	5	31
IUGR	2	1	50	1	50
PPROM	11	3	27.3	4	36
C.S	17	0	0	2	11.8
DM	10	0	0	0	0

- The above table shows predictability of RDS by LBC in each high risk group.
- In different high risk group the LBC predicts RDS differently. There is no Statistical correlation, this is because gestational age is not standardized in each group.

**TABLE 11. SEX OF THE BABIES**

<b>Sl.No</b>	<b>Sex</b>	<b>Number of Babies</b>	<b>% of Patients</b>	<b>Number of cases of RDS</b>	<b>% of RDS</b>
1	Male	59	59	11	18.6
2	Female	41	41	7	17

- 59% of the babies were male
- Out of 18 cases of RDS, 11 babies were male.
- There was no statistically significant difference in the incidence of RDS between male and female babies.(p value=0.8)

**TABLE 12.BIRTH WEIGHT OF THE BABIES**

<b>Sl.No</b>	<b>Birth Weight (kg)</b>	<b>Number of Babies</b>	<b>% of Babies</b>	<b>Number of cases of RDS</b>	<b>% of RDS</b>
1	$\leq 2$	25	25	10	40
2	2.1-2.5	32	32	8	25
3	2.6-3.0	30	30	-	-
4	3.1-3.5	9	9	-	-
5	$\geq 3.6$	4	4	-	-

- In this study, 75% of the babies had birth weight above 2kg (Most salvageable birth weight).
- In this study RDS occurred only in babies with birth weight less than 2.5kg.
- There was a statistically significant difference in the occurrence of RDS among the babies with different birth weights.(p value <0.001)

**TABLE 13. ASSESSMENT OF GESTATIONAL AGE AFTER  
BIRTH OF BABIES**

<b>Sl.No</b>	<b>Gestational age of Babies (weeks)</b>	<b>Number of Babies</b>	<b>% of Babies</b>	<b>Number of cases of RDS</b>	<b>% of RDS</b>
1	Term	19	19	0	0
2	Pre Term	62	62	12	19
3	Very Pre Term	19	19	6	31.6

- Nearly 80% of the babies were pre term and 19% of the babies were very pre term.
- Occurrence of RDS was very high in very pre term infants.
- There was a statistically significant difference in the occurrence of RDS among the different gestational age group(p value <0.05)



**TABLE 14. RESPIRATORY DISTRESS SYNDROME (n=18)**

<b>Sl.No</b>	<b>LBC / <math>\mu</math>l</b>	<b>Number of Babies developed RDS</b>	<b>Number of Babies died</b>
1	> 50,000	3	0
2	< 50,000	15	7

- In this study, the incidence of RDS was 18%. Among 18 babies, 3 of those developed RDS had LBC count above 50,000 $\mu$ l and 15 of those had LBC below 50,000 $\mu$ l. Death due to RDS occurred only in those with LBC less than 50,000 $\mu$ l.

## STATISTICAL ANALYSIS

### PREDICTION OF RDS

Statistical attributes of lamellar body count as a screening test for fetal lung maturity was analyzed and found as follows:

The main observation in this study was the presence or absence of RDS in the neonate. If death had occurred the same was recorded.

Lamellar body count above 50,000/ $\mu$ l was considered as a positive test, which indicates fetal lung maturity. A negative test result means lamellar body count of below 50,000/ $\mu$ l.

**TABLE 15. ASSOCIATION BETWEEN LBC AND RDS**

<b>LBC/<math>\mu</math>l</b>	<b>NO RDS (Mature lung)</b>	<b>RDS (Immature lung)</b>
> 50,000	74	3
< 50,000	8	15

### TRUE POSITIVE

Those individuals found positive on the test had mature fetus and the fetus did not develop RDS.

$$a = 74$$

## FALSE POSITIVE

This denotes who had the positive test results but the fetus developed RDS.

$$\mathbf{b = 3}$$

## FALSE NEGATIVE

Denotes those with negative results but did not develop RDS.

$$\mathbf{c = 8}$$

## TRUE NEGATIVE

This denotes negative test results with out RDS.

$$\mathbf{d = 15}$$

## SENSITIVITY

Ability of the test to identify correctly all those have mature lung, that is do not develop RDS.

$$a*100/(a+c) = 74*100/(74+8) = \mathbf{90\%}$$

## SPECIFICITY

Ability of the test to identify correctly who do not have mature lung, that is those who do not develop RDS.

$$d*100/(b+d) = 15*100/(3+15) = \mathbf{83\%}$$

## POSITIVE PREDICTIVE VALUE

This reflects the diagnostic power of the test. Predictive value of the positive test indicates the probability of getting mature fetus with a positive results.

$$a*100/(a+b) = 74*100/(74+3) = \mathbf{96\%}$$

## NEGATIVE PREDICTIVE VALUE

It denotes the probability of getting immature fetus with a negative test.

$$d*100/(c+d) = 15*100/(8+15) = \mathbf{65\%}$$

## TEST OF SIGNIFICANCE

Chi-Square Test

$$\chi^2 = \sum ((O - E)^2/E)$$

Applying values of O and E from the above table,

Degrees of freedom: 1

Chi-square = 45.1185082150087

Since  $p$  is less than or equal to 0.001, the distribution is significant.

There is strong correlation between Screening test and fetal lung maturity.

## **RELATIONSHIP BETWEEN GESTATIONAL AGE & LBC.**

Applying values of Gestational age & LBL in the correlation co-efficient ( $r$ ) formula, the value of +1 indicates a strong positive association between Gestational & LBC.

## **DISCUSSION**

This one year prospective clinical outcome study was carried out among the 100 pregnant women in Govt. Kasturba Gandhi hospital, Chennai to evaluate the efficacy of amniotic fluid lamellar body count (LBC) as a screening test for fetal lung maturity and the results are discussed as follows.

### **Demographic characters (Table 1, 2, 3, 4)**

- In this study majority of the patients were in the age group of 21-30yrs, which accounts for 83%, and none of the patients were above 35yrs.
- 95% of the patients were booked.
- Majority of the patients belonged to class V socio economic class.
- Primi contributes to 52% of the study population, 2<sup>nd</sup> and 3<sup>rd</sup> gravida accounts for 44% and the 4<sup>th</sup> gravida and above accounts for only 4%.

The Demographic factors showed no statistically significant difference in homogeneity between the LBC <50,000/ $\mu$ l and >50,000/ $\mu$ l

### **Risk factors and mode of delivery (Table 5, 7)**

- Various risk factors for RDS which were present in the mothers selected for this study were depicted in these tables.. 44% of the patients had preterm labor without any other complications, 17% cases of repeat cesarean section

cases were also included in the study. Other risk factors included were PET, Diabetes complicating pregnancy and PPRM.

- In this study 2/3 of the patients were delivered by labor natural and 1/3 by cesarean section. Even though cesarean section is a risk factor for the occurrence of RDS, mode of delivery did not significantly affect the occurrence of RDS (p=0.8)

#### **Incidence of RDS (Table 6, 12, 13)**

- The incidence of RDS in this study was 18% which is slightly on the higher side when compared to 12% reported by *Carlos et al*<sup>17</sup>, 14% reported by *Fakhory et al*<sup>39</sup> and 11% reported by *Ahwood et al*<sup>16</sup>.
- This is probably due to high proportion of (19%) very preterm (<32weeks) birth in this study when compared to 11% in *Carlos et al* study.
- In this study 15 out of 18 RDS cases had been correctly predicted which is comparable to Khazardoost et al<sup>13</sup> study where 17 out of 20 RDS cases had been predicted correctly.
- Out of 18 cases of RDS, 7 babies died due to RDS.
- Case fatality rate was 28%
- Death due to RDS had occurred only in the group of patients who had LBC <50,000/ $\mu$ l

### **Table (10, 11, 12)**

- In this study the incidence of RDS was not significantly affected by the sex of the babies.
- The incidence of RDS was high in very preterm infant and babies with birth weight <2kg. since LBC correlates well with gestational age, it indirectly correlates with the birth weight also.

### **LBC and its validity**

- In this study, the lamellar body count varied from 5,000/ $\mu$ l to 2,88,000/ $\mu$ l with a mean of 86,378/ $\mu$ l which is comparable to *Carlos et al* study, in that LBC ranged from 4000/ $\mu$ l to 3,29,000/ $\mu$ l. In 23% of patients LBC was below 50,000/ $\mu$ l
- To study impact of the gestational age on the test performance, study group patients were divided into three groups based on four weeks gestational age intervals.

*Lee IS et al*<sup>12</sup> study proved that the LBC correlated positively with advancing gestational age.

- The percentage of LBC <50,000/ $\mu$ l in the gestational age group of 28-31 weeks was 55.6% against 22.8% in the gestational age group of 32-35 weeks and all had LBC above 50,000/ $\mu$ l in the gestational age group of 36-40 weeks.

- *Carlos et al* calculated the sensitivity, specificity, positive predictive value and negative predictive value for each gestational age group separately and found that these values were equal to or better than that for either L/S ratio or the phospholipids profile.
- In the critical function of predicting fetal lung maturity, our data demonstrated that when using a cut off of 50,000/ $\mu$ l, lamellar body count showed 90% sensitivity, 83% specificity, 96% positive predictive value and 65% negative predictive value. *p* value is <0.001 which is statistically significant.

These values are comparable to other studies.

STUDY	LBC CUT OFF VALUE	SENSITIVITY %	SPECIFICITY %	PPV %	NPV %
<i>Carlos et al</i> <sup>17</sup>	30,000	100	72	92	100
<i>Ashwood et al</i> <sup>16</sup>	55,000	95	87	98	75
<i>Lee Is et al</i> <sup>12</sup>	50,000	100	80	-	-
<i>Khazardoost et al</i> <sup>13</sup>	50,000	85	70	48	93
<i>Lewis PS et al</i> <sup>18</sup>	32,000	96	98	99	63
<i>Neerhof MG et al</i> <sup>26</sup>	50,000	95	88	65	98
<i>Dalence CR et al</i> <sup>27</sup>	35,000	85	98	99	63



- The negative predictive value of 65% can be improved by lowering the cut off value for predictive lung immaturity. *Carlos et al* recommended a cut off of 10,000/ $\mu$ l to predict a high likelihood of pulmonary immaturity.
- Ashwood et al<sup>16</sup> recommended a cut off value of 15,000/  $\mu$ l to predict pulmonary immaturity.

### **LAMELLAR BODY COUNT IN DIABETIC PATIENTS**

- The lamellar body counts on the samples from the 10 diabetic mothers were studied. All had count above 50,000/ $\mu$ l and none had developed respiratory distress syndrome. *De Roche ME et al*<sup>20</sup> reported that a lamellar body count of 37,000/ $\mu$ l correlated with LS ratio and phosphatidyl glycerol value in the pregnancies of diabetic patients. However in this study the sample size and the period of gestation were inadequate to analyse the results of lamellar body count in diabetic mothers and it needs a larger study with adequate sample size.

### **ANTENATAL STEROIDS**

- Patients who received antenatal steroids and delivered after treatment were excluded from the study. 12 patients received steroids and delivered within 24hrs of first dose. They were included in the study. 5 out of 12 babies developed respiratory distress syndrome, all had lamellar body count <50,000/ $\mu$ l.

## **LIMITATIONS AND LACUNAE**

1. In this study lower cut off value of predicting lung immaturity was not standardized. The cut off value of 50,000 was taken following the overall average in various studies. Only few authors have specifically reported a lamellar body count value below which the risk of immaturity is exceedingly high. However no one has made recommendations for cut off below which further testing would be necessary.<sup>16</sup>
2. Even though the cell counter is available in many institutions, private laboratories and private hospitals, the use of amniotic fluid in the routine hematology analyzer needs manufacture's explanation and recommendations.
3. In this study lamellar body count is not compared with the gold standard L/S ratio because since it is not easily available.

## SUMMARY

This prospective clinical outcome study was undertaken in Government Kasturba Gandhi Hospital, Chennai during the period of 2005-2006 among the 100 pregnant women between 28 and 40 weeks of gestation, with the risk factors for RDS, to prove the efficacy of amniotic fluid lamellar body count as a screening test for fetal lung maturity.

The various observations are:

1. The demographic factors studied were age, booking status, socio economic status, and gravidity. They showed no statistically significant difference in homogeneity between the LBC  $<50,000$  and  $>50,000/\mu\text{l}$ .
2. Mode of delivery did not significantly affect the occurrence of RDS.
3. In this study the incidence of RDS was 18%. In all cases of severe RDS the count was below  $50,000/\mu\text{l}$ . Case fatality rate was 28%. Death due to RDS had occurred only in group of patients who had LBC below  $50,000/\mu\text{l}$ .
4. In this study LBC ranged from  $5,000/\mu\text{l}$  to  $2,88,000/\mu\text{l}$ . LBC correlates well with the gestational age, as the gestational age increase the LBC also increases.

5. In predicting fetal lung maturity, the LBC showed 90% sensitivity, 83% specificity, 96% positive predictive value and 65% negative predictive values which are comparable to many other studies.
6. The sample size and the duration of gestation were inadequate in this study to analyze the effect of LBC in diabetic patients.

## **CONCLUSION**

Management of pregnancies at risk for the development of neonatal RDS would be enhanced by a rapid, accurate, and objective test for fetal lung maturity. In most clinical settings, the most important function of a fetal lung maturity test is to predict accurately the absence of RDS.

In many studies lamellar body count compares favorably with traditional phospholipids testing and L/S ratio in the prediction of fetal lung maturity.

Our data indicates that using a LBC cut off of 50,000/ $\mu$ l to predict fetal lung maturity, the lamellar body count showed 90% sensitivity, 83% specificity, 96% positive predictive value and 65% negative predictive value.

The LBC has many advantages over gold standard L/S ratio. LBC is faster, more precise, more objective, inexpensive, requires smaller sample volume and it is not invalidated by the presence of lysed blood or meconium. In addition the instrumentation required for the test is almost universally available, allowing it to be performed in laboratories where L/S ratio or traditional phospholipid analysis is not available.

Since LBC is cost effective, can be performed quickly and the efficacy is also acceptable high, LBC may be used as the test of choice in the assessment of fetal lung maturity.

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# PROFORMA

## AMNIOTIC FLUID LAMELLAR BODY COUNT AS A SCREENING TEST FOR FETAL LUNG MATURITY

Sl. No:

**NAME :**

**AGE :**

**IP.No:**

**Unit:**

D.O. Admission:

Booked/Unbooked:

D.O. Delivery:

Immunized /Unimmunized:

D.O. Discharge:

Occupation:

Income:

Address:

OBSTETRIC FORMULA:

LMP :

E.D.D:

M/S :

M/H :

OBSTETRIC HISTORY – RISK FACTORS:

Preterm / DM / PET / PROM / LSCS

PAST MEDICAL / SURGICAL HISTORY:

FAMILY HISTORY:

## EXAMINATION:

Ht : Wt :

Pallor : Edema:

PR : BP :

CVS : RS :

## ABDOMINAL EXAMINATION

Uterus (Fundal height): SFH :

Acting / Not acting : EFW :

Presentation : Liquor:

FHR :

P/V:

## INVESTIGATIONS:

Hb% VDRL

Urine Alb : HIV

Sugar :

Blood Sugar: Hbs Ag

Blood grouping & Typing

USG:

1<sup>st</sup> Trimester Scan

Present Scan

LAMELLAR BODY COUNT

LABOR

Mode of delivery:

Intra partum Complications:

NEONATAL DETAILS

Sex :

GA:

B.Wt:

Condition at Birth:

Apgar:

1 mt:

5 mt:

10 mt:

DISC NO:

DELIVERED ON:

MORBIDITY:

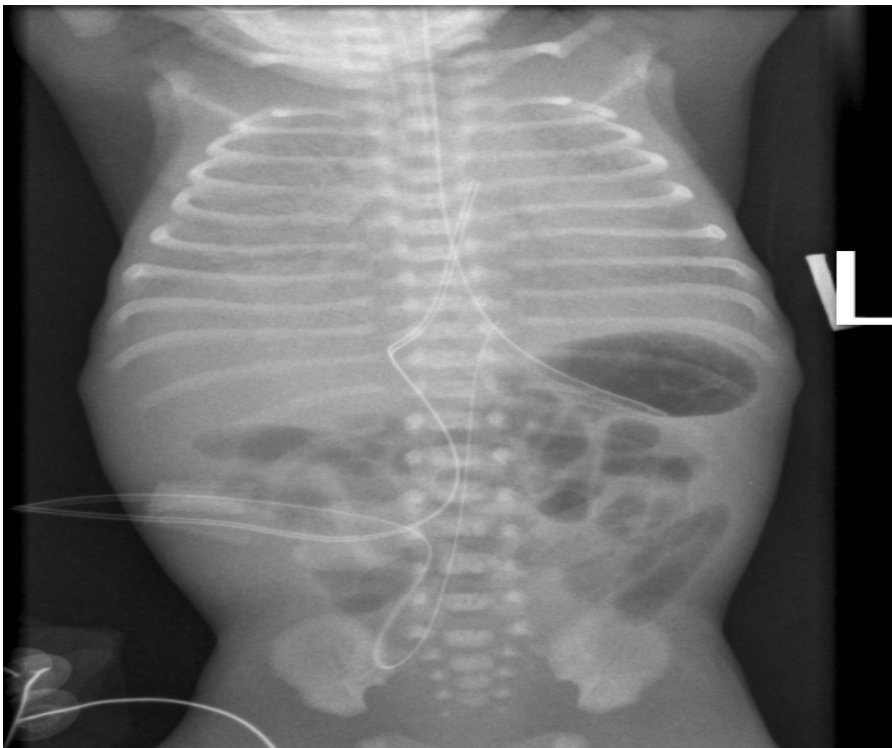
NICU Admission:

Asphyxia/RDS/Sepsis/Jaundice/Apnoea/Seizure/Bronchopneumonia/NEC

MORTALITY: Still Birth / Neonatal death - Cause of death



**X – Ray pictures of neonate with RDS showing diffuse ground glass” appearance”**





SYSMEX – CELL COUNTER



Preterm Neonate with RDS





Lamellar bodies in the cytoplasm of type II Cells.

						MASTER CHART				BABY DETAILS					
Sl. No	NAME/IP NO.	Age	B/UB	SES	Gravidity	Risk Factor	GA	Mode of delivery	LBC	Sex	B.Wt	GA	Apgar	RDS Scoring	Mortality
1	PADMAPRIYA-6148	21	B	V	P	PT	35	LN	75000	M	2.6	PT	>7		
2	POORNIMA-6169	21	B	IV	P	PT	33	LN	64000	F	2.2	PT	>7		
3	KAVITHA-6187	24	UB	V	G2	PET	34	LN	79000	F	2.8	PT	>7		
4	ANJUGAM-6228	28	B	V	G3	C.S	39	EM.C.S	152000	M	3.4	T	>7		
5	ABIRAMI-6168	22	B	V	G2	PT	31	LN	50000	M	1.9	VPT	>7		
6	FATHIMA-6304	18	B	V	P	PPROM	32	LN	24000	M	2	PT	5-7	mild RDS	
7	NIRMALA-6158	21	B	V	P	PT	31	LN	48000	M	2.2	VPT	>7		
8	MANJULA-6256	33	B	V	G5	C.S	40	EM.C.S	206000	M	4	T	>7		
9	UMA-6345	23	B	V	G2	DM	35	LN	68000	F	2.5	PT	>7		
10	ASWINI-6189	26	B	IV	G3	IUGR	34	LN	72000	M	1.8	PT	>7		
11	BALA-6237	19	B	V	P	PET	35	LN	106000	F	2.8	T	>7		
12	GOMATHY-6543	29	UB	V	G4	PT	33	LN	46000	M	2.3	PT	5-7	mild RDS	
13	NALINI-6431	27	B	V	G2	PPROM	34	EM.C.S	86000	F	2.9	PT	>7		
14	JOTHY-6447	24	B	V	G2	GDM	37	FORCEPS	172000	M	3.6	PT	>7		
15	ANBU-6789	22	B	V	P	PT	30	LN	52000	M	1.8	VPT	>7		
16	PARIMALA-6679	20	B	V	P	PT	35	LN	73500	M	2.7	PT	>7		
17	DHAVAMANI-6810	23	B	V	P	PET	34	EM.C.S	42000	F	2.2	PT	5-7	mild RDS	
18	MAHADEVI-7010	24	B	III	G2	PT	32	LN	55000	M	2.5	VPT	>7		
19	SAHIRA-7202	27	B	V	G3	PT	34	LN	140000	F	3.1	PT	>7		
20	LATHA-7148	24	B	V	P	C.S	40	EL.C.S	182000	F	3.2	T	>7		
21	PADMAVATHY-7156	20	B	V	P	C.S	39	EM.C.S	189000	M	3	T	>7		
22	PRAMILA-7246	27	B	V	G2	PT	33	LN	58000	M	2.2	PT	>7		
23	DEVIKA-7237	22	UB	V	P	PET	35	LN	78000	F	2.6	PT	>7		
24	VANAJA-7317	23	B	V	P	PET	34	LN	42000	M	2.1	PT	5-7	mild RDS	
25	RAMYA-7287	22	B	IV	P	PT	30	LN	59000	F	1.8	VPT	>7		
26	SATHYA-7295	21	B	V	P	PT	35	FORCEPS	86000	M	2.5	PT	>7		

Sl. No	NAME/IP NO.	Age	B/UB	SES	Gravidity	Risk Factor	GA	Mode of delivery	LBC	Sex	B.Wt	GA	Apgar	RDS Scoring	Mortality
27	ANJAL-ANNE-7456	26	B	V	G3	GDM	40	VACUUM	82500	M	3.2	T	>7		
28	CHANDRA-7689	18	B	V	P	PT	34	LN	39000	M	2.1	PT	<5	severe RDS	+
29	CATHERINE-7897	26	B	IV	G2	C.S	38	EL.C.S	192000	F	2.8	T	>7		
30	KOTHAI-7793	24	B	V	P	PPROM	30	LN	32000	F	1.6	VPT	<5	severe RDS	+
31	NITHYA-7987	23	B	V	P	PT	31	LN	56000	M	1.8	VPT	>7		
32	MOHANA-8002	26	UB	V	G3	PET	35	EL.C.S	72000	F	2.3	PT	>7		
33	SOPHIA-8006	20	B	V	P	PT	34	LN	69000	M	2.5	PT	>7		
34	SUDHA-7995	26	B	V	G3	C.S	38	EL.C.S	278000	F	2.8	T	>7		
35	REVATHY-8018	23	B	V	P	GDM	38	LN	288000	F	3	T	>7		
36	SASI-8216	22	B	V	G2	PT	33	LN	33000	M	1.6	PT	>7		
37	AMBIKA-8218	21	B	V	P	PPROM	35	EM.C.S	64000	F	2.3	PT	>7		
38	SELVI-8217	21	B	V	P	PT	31	LN	23000	F	2.3	VPT	>7		
39	KUMUDHA-8246	24	B	IV	P	PT	35	LN	135000	M	2.9	PT	>7		
40	LAKSHMI-8300	27	UB	V	G2	PT	33	LN	82000	M	2.8	PT	>7		
41	HAJIRA-BANU-8433	26	UB	V	G2	C.S	35	EL.C.S	96800	F	2.7	PT	>7		
42	VIJAYA-8480	32	B	V	G3	PT	32	LN	48000	F	2.3	PT	>7		
43	SANGEETHA-8565	26	B	III	P	C.S	37	EM.C.S	182000	M	3.1	PT	>7		
44	KAVITHA-8569	19	B	V	P	PT	35	EM.C.S	36000	F	2.1	PT	5-7	mild RDS	+
45	THAYAMMAL-8600	21	UB	V	P	PET	34	LN	76000	M	2.6	PT	>7		
46	BAGYAM-8720	22	B	V	P	PET	35	LN	52000	M	2.1	PT	>7		
47	MAHESWARI-9208	22	B	IV	G2	PT	31	LN	50000	M	2	VPT	>7		
48	KALIAMMAL-9246	26	B	V	G2	GDM	38	LN	179000	F	3	T	>7		
49	BAIRAVI-9925	23	B	V	P	C.S	34	EL.C.S	65000	M	2	PT	5-7	mild RDS	
50	RANI-10026	22	B	V	P	PT	35	LN	52000	F	2.6	PT	>7		
51	SARADHA-10224	19	B	V	P	PT	31	LN	54000	M	1.4	VPT	>7		
52	PANGAJAM-10986	21	B	V	P	PPROM	35	LN	122000	F	2.1	PT	>7		
53	SUSHILA-10046	31	B	V	P	GDM	35	EM.C.S	93800	F	3.6	PT	>7		

Sl. No	NAME/IP NO.	Age	B/UB	SES	Gravidity	Risk Factor	GA	Mode of delivery	LBC	Sex	B.Wt	GA	Apgar	RDS Scoring	Mortality
54	DEIVANAI-11026	28	B	IV	G3	PPROM	34	EM.C.S	220000	M	2.25	PT	>7		
55	RAMANI-11463	24	UB	V	G2	PT	34	FORCEPS	66000	M	2.1	PT	>		
56	PONNAMMAL-11256	23	B	V	G2	PET	32	LN	38000	M	1.8	PT	<5	severe RDS	+
57	SUMATHY-11098	26	B	V	G3	PPROM	35	LN	78000	M	2.25	PT	>7		
58	PREMA-008	29	B	V	G2	PET	35	LN	72000	F	2.1	PT	>7		
59	SHANTHA-026	23	B	V	G2	PT	32	EM.C.S	33000	M	1.9	PT	>7		
60	RADHIKA-073	21	B	V	P	PT	34	LN	96000	M	2.6	PT	>7		
61	JOTHY-146	27	UB	V	G2	PT	34	LN	58000	M	2.6	PT	>7		
62	GEETHA-722	26	B	V	P	PET	30	LN	40000	F	2.2	VPT	>7		
63	KARPAGM-906	22	B	IV	P	PT	35	LN	178000	M	2.8	PT	>7		
64	SUJI-1028	18	B	V	P	PET	31	LN	23000	M	1.6	VPT	5-7	mild RDS	
65	VEDHA-1543	24	B	V	G2	C.S	37	EM.C.S	82000	F	2.2	PT	5-7	mild RDS	
66	SAMSATH-2037	22	UB	V	P	PT	31	LN	36000	M	1.6	VPT	5-7	mild RDS	
67	RUBA-2249	21	B	V	P	C.S	40	EL.C.S	98000	F	3.6	T	>7		
68	DEEPA-2878	26	B	V	G3	PT	34	LN	84000	M	2.4	PT	>7		
69	SOUNDARAM-3040	18	B	IV	P	PPROM	35	LN	92000	F	2.8	PT	>7		
70	VASANTHI-3725	20	B	V	P	PT	33	LN	38000	F	2.8	PT	>7		
71	ASHA-4023	22	B	III	P	DM	36	EM.C.S	85000	M	3	PT	>7		
72	CHELLAM-4218	28	B	V	G2	GDM	39	LN	93600	M	3.4	T	>7		
73	KARUPPAYI-4137	21	B	V	G2	PT	35	LN	68000	F	2.2	PT	>7		
74	RADHA-4173	31	B	V	G2	PET	28	LN	8000	M	1	VPT	<5	severe RDS	+
75	MARY-4256	21	B	V	P	PPROM	36	LN	184000	M	2.8	PT	>7		
76	PARVEEN-4448	25	B	IV	P	PT	37	FORCEPS	126000	F	2.75	PT	>7		
77	SUGANTHI-4256	26	B	V	G3	IUGR	34	LN	45000	M	1.6	PT	<5	severe RDS	+
78	SARASU-4789	29	B	V	G4	C.S	40	EL.C.S	134000	F	3.25	T	>7		
79	KASTHURI-4926	26	B	V	G3	PT	35	LN	110000	M	2.8	PT	>7		
80	USHA-4898	24	B	V	G2	C.S	39	EL.C.S	152000	M	3.2	T	>7		

Sl. No	NAME/IP NO.	Age	B/UB	SES	Gravidity	Risk Factor	GA	Mode of delivery	LBC	Sex	B.Wt	GA	Apgar	RDS Scoring	Mortality
81	BANU-5125	23	B	V	G2	PT	29	LN	32000	F	1.6	VPT	5-7	mild RDS	
82	KAMATCHI-5278	26	B	V	G3	PT	32	LN	58000	M	2.1	PT	>7		
83	THILAGA-5254	24	B	IV	G2	C.S	38	EL.C.S	92000	M	3.2	T	>7		
84	SAVITHA-5310	26	B	V	G2	GDM	37	EM.C.S	120000	M	3.1	PT	>7		
85	VALLI-5355	20	B	V	P	PT	35	EM.C.S	68000	M	2	PT	>7		
86	DHANAM5446	23	UB	V	G2	C.S	40	EL.C.S	86000	F	3	T	>7		
87	MUKKAYI-5567	22	B	V	G3	C.S	38	EM.C.S	82000	M	2.8	T	>7		
88	KANNAGI-5569	32	B	V	P	PT	28	LN	6600	F	1.1	VPT	<5	severe RDS	+
89	FATHIMA-5800	21	B	V	G4	PPROM	34	LN	52000	M	1.8	PT	5-7	mild RDS	
90	PATCHI-5925	28	B	V	P	PET	34	LN	88000	M	2.6	PT	>7		
91	FLORENCE-5968	26	B	III	G2	PT	30	LN	50000	F	1.8	VPT	>7		
92	MARIAMMAL-6120	28	B	V	G3	PT	31	LN	50000	M	2	VPT	>7		
93	PRABHA-6134	18	B	IV	P	C.S	40	EL.C.S	135000	F	3	T	>7		
94	JEYANTHI	24	UB	V	P	PPROM	35	EM.C.S	42000	F	2.4	PT	5-7	mild RDS	
95	VADIVU-6258	24	B	V	P	PT	30	LN	36000	M	1.8	VPT	>7		
96	SHEELA-6145	21	B	IV	G2	GDM	38	VACUUM	140000	F	3.2	T	>7		
97	SAROJA-6334	22	B	V	P	PT	35	LN	58000	M	2.6	PT	>7		
98	SHANTHI-6447	28	B	V	P	PET	34	LN	96000	M	2.25	PT	>7		
99	CHITHRA-6578	21	B	V	P	PT	32	EM.C.S	86000	F	2.2	PT	>7		
100	GOMATHY-6876	22	B	V	P	PT	33	LN	66000	M	2.1	PT	>7		
	B - Booked				DM - Diabetes Mellitus				T - Term						
	UB - Unbooked				GDM - Gestational Diabetes Mellitus				PT - Preterm						
	SES - Socio Econmic Status				C.S - Cesarean Section				VPT - Very Preterm						
	P - Primi				EL.C.S - Elective Cesarean Section				LN - Labour Natural						
	GA - Gestational Age				EM.C.S - Emergency Cesarean Section				PET -- Preeclamptic toxemia						

## 1. AGE DISTRIBUTION

